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Abstract

Genomic instability, evidenced by non-clonal chromosomal abnormalities, delayed death, and increased recombination, is increased in the progeny of irradiated cells. We have shown that radiation exposed non-malignant human mammary epithelial cells undergo aberrant acinar morphogenesis when suspended in a basement membrane type matrix. The loss of cell-cell adhesion, down-regulation of E-cadherin and gap junctions, and perturbed integrin expression shown by irradiated cells is consistent with neoplastic progression. In the current proposal, we wish to test the hypothesis that persistent disruption of extracellular signaling in irradiated cells promotes genomic instability. We plan to measure centrosomes, chromosome number and aneuploidy in the daughters of irradiated cells, determine the dose dependence, and how transforming growth factor beta, which augments the morphogenic disruption, affects genomic instability. We will also test if the radiation phenotype can be transmitted to unirradiated cells and whether its prevalence in irradiated cells is epigenetic in nature. These exploratory studies will define non-mutational mechanisms by which ionizing radiation, a known carcinogen of human breast, affects carcinogenesis.

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HYPOTHESIS AND OBJECTIVES

The first important implication of these data is that radiation exposure of individual cells leads to a persistently altered phenotype in daughter cells. The second is that this phenotype lacks critical controls imposed by the microenvironment to maintain tissue integrity. Our hypothesis is that the disruption of morphogenesis releases constraints on genomic integrity in preneoplastic cells. Thus we predict that colonies arising from irradiated cells will show increased genomic instability *due to the lack of microenvironment control* rather than as a direct result of DNA damage. In the current IDEA grant, we seek to determine whether such a correlation exists. In addition, to be in a position to manipulate the phenotype in future studies, we wish to determine the underlying basis for the irradiated phenotype. In aim 2, we will determine whether irradiated cells can communicate their phenotype in a fashion similar to the 'bystander effect' that occurs when unirradiated cells respond as if they were irradiated under conditions of heterogeneous radiation exposure [1,2]. In aim 3, we will begin studies to test an alternative or additional mechanism in which the phenotype is perpetuated by epigenetic modifications leading to altered gene expression. Together these studies will test whether the irradiated HMEC phenotype contributes to radiation-induced genomic instability that is observed in cells generations after radiation exposure.

AIM 1 Determine whether the progeny of irradiated cells have increased indices of genomic instability. Confocal microscopy and image analysis will be used to quantify features of the irradiated cell phenotype, which will be correlated with three indices of genomic instability: DNA ploidy, centromeric FISH and centrosome integrity.

AIM 2 Determine whether the irradiated cell can communicate its phenotype via extracellular signaling. If so, unirradiated cells will be influenced to alter their cell-cell interactions via a bystander mechanism. We will use 3D microscopy to study the establishment of intercellular contacts in radiation chimeric colonies formed in the rBM assay.

AIM 3 Test the hypothesis that epigenetic molecular mechanism perpetuate the irradiated phenotype from generation to generation of human mammary epithelial cells. E-cadherin expression is frequently suppressed by epigenetic mechanisms in cancer and is radically decreased following radiation. We will test the role of DNA methylation and/or histone deacetylation in regulation of the irradiated E-cadherin phenotype.

Body

In both women and rodents, exposure to high dose ionizing radiation represents a well-established carcinogen. Epidemiologic data demonstrates that there is a significantly increased risk of breast cancer in women exposed to as little as 1 Gy as a result of atomic bomb [3], therapeutic [4,5] or diagnostic [6] radiation exposures. We have previously proposed that the cell biology of irradiated tissues is indicative of a coordinated multicellular damage response program in which individual cell contributions are directed towards repair of the tissue [7]. In both murine and human models of breast cancer, our studies indicate that radiation exposure modifies the microenvironment in a manner that can promote malignant progression. Our global hypothesis is that extracellular signaling and multicellular responses following radiation contribute to its carcinogenic potential.

We have demonstrated that colonies arising from irradiated HMEC exhibit a consistent phenotype consisting of inappropriate intercellular adhesion, deranged extracellular adhesion molecules, loss of gap junction proteins, and disorganized tissue-specific organization [8]. This phenotype is augmented by the presence of TGF-β, which itself is rapidly and persistently activated in irradiated tissue [9]. These data are remarkable in that the phenotype is inherited by the daughters of individually irradiated cells, suggesting that radiation induces a heritable derangement of pathways affecting cell adhesion, ECM interactions, epithelial polarity and cell-cell communication.

We proposed that these radiation-induced changes in cell-cell and cell-ECM interactions are consistent with malignant progression. If so, we asked whether genomic stability was similarly compromised. To do so, we began investigations to determine the status of centrosomes in this model. Centrosomes are tiny organelles that contain discrete protein aggregates that nucleate microtubule growth, organize spindle functions, and provide docking sites for protein complexes involved in cell cycle progression, checkpoint control and epithelial cell polarization (reviewed in [10]). Centrosomes are abnormal in both number, size and distribution in many solid tumors [11]. Abnormal centrosomes result in abnormal chromosome segregation and aneuploidy. Two models of centrosome involvement in cancer are currently debated. The first holds that centrosome abnormalities, like chromosome aberrations, are the product of genomic instability; the second is that centrosome abnormalities drives genomic instability. The latter model is consistent with recent report that abnormal centrosomes precede morphological changes in transformation by HPV E7 oncoprotein [12] and that overexpression of pericentrin, a component of centrosomes, induces chromosome instability in prostate cancer cells [11].

Key Research Accomplishments

Task 1: To determine the frequency of genomic instability in clonal colonies arising from sham-irradiated, TGF-β treatment, irradiated, and irradiated, TGF-β treated cells.

- Replicated radiation dose response of induced centrosome abnormalities in HMT3522 S1 HMEC (n=3).
- Demonstration that MCF10A HMEC also show radiation dose response of centrosome abnormalities (n=3).
- Demonstrated that exogenous TGFβ suppresses abnormal centrosome induction by radiation.
- Demonstrated that neutralizing antibody to TGFβ increases the frequency of centrosome abnormalities in both control and irradiated HMEC.
- Demonstrated that keratinocyte cell lines from TGFβ1 knockout mice have high levels of centrosome abnormalities compared to TGFβ1 heterozygote cells.
- Demonstrated that abnormal centrosomes are increased following passage of irradiated cells, which indicates that this population is survival competent.

We discovered that centrosome aberrations are an early and persistent feature of irradiated HMEC and have focused on the regulation of their frequency. These studies were carried out on HMEC grown on tissue culture plastic rather than embedded in Matrigel since we have shown in complementary studies that the radiation-induced phenotype is evident in monolayer as well (Erickson and Barcellos-Hoff, unpublished data). HMEC were seeded onto chamber slides. On day 0, the cells were irradiated within 4 hours of plating using ^{60}Co γ-radiation and harvested at day 6 for staining γ-tubulin, a centrosome component. After immunofluorescence images were acquired

using a CCD camera, the centrosome status of the cells was determined by manual counting of digital images. Cells were categorized as having abnormal centrosomes when their centrosomes were irregular in size, shape or number (>3 centrosomes per cell). We completed 3 replicates of the dose response in HMT3522 S1 and MCF-10A HMEC (Figure 1A). To ask whether centrosome amplification in irradiated cells is persistent MCF-10A cells treated with +/-200cGy were trypsinized and replated. Assessment of centrosome status by γ-tubulin immunofluorescence showed that IR cells continued to have increased frequency of abnormal centrosomes compared to control cells.

Our hypothesis that disrupted morphogenesis is accompanied by genomic instability is supported by the finding of centrosome abnormalities in the daughters of irradiated cells. We showed that exogenous TGFβ augments the radiation-induced transition from epithelial to mesenchymal phenotype in terms of 3-dimensional morphogenesis [Park, 2003] and in the monolayer culture model (unpublished data). Thus, we asked whether addition of TGFβ would

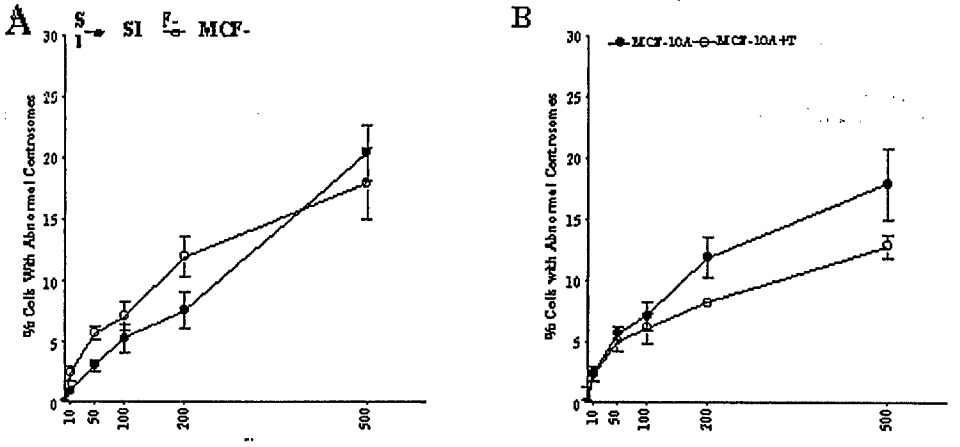

Figure 1. HMECs were irradiated to the indicated dose shortly after plating, grown under standard conditions and fixed at day 6. **(A)** S1 (closed circles) and MCF-10A (open circles) **(B)** MCF-10A cells mgrown in the absence (closed) or presence of 400 pg/ml TGF-β (open circles).

increase instability. Studies carried out in S1 and MCF-10A cells show that when cells are grown in the presence of TGFβ the number of cells containing abnormal centrosomes is reduced, which suggests that exogenous TGFβ actually protects both S1 and MCF10A HMEC from radiation induced genomic instability (Figure 1 B).

We have not found any literature that previously established a link between TGFβ and the centrosome. These data suggests that TGFβ safeguards cells from genomic instability by protecting them from centrosome abnormalities. Further conformation of this idea was seen when we examined the centrosome status in keratinocytes isolated from mice homozygous or heterozygous for the TGFβ1 gene; *Tgfβ1* null keratinocytes have more abnormal centrosomes than TGFβ +/- keratinocytes (Figure 2b).

Our studies show that the disruption of extracellular interactions occurs in almost all colonies formed in Matrigel by cells that survive irradiation [Park, 2003]. If the mechanisms by which the irradiated phenotype is perpetuated involve extracellular signaling via soluble molecules or cell contact, irradiated cells will be able to influence unirradiated cells via a "bystander" mechanism.

We have now shown that TGFβ is clearly implicated. We first showed that TGFβ is activated in vivo after exposure to ionizing radiation [13]. We have now found that irradiated S1 cells also increase TGFβ signaling. We hypothesized that this radiation-induced TGFβ activity is acting in a bystander capacity to protect irradiated cells from genomic instability based on the data from irradiated HMEC treated with exogenous TGFβ. Pan-specific TGFβ neutralizing antibodies were used to test whether the endogenous TGFβ affects the number of cells containing abnormal centrosomes. S1 cells were treated with +/- 200 cGy and grown in the presence of TGFβ blocking antibodies or IgG controls. Interestingly, blocking TGFβ signaling affected unirradiated and irradiated cells equally, i.e. both treatment groups displayed an increase in abnormal centrosomes (Figure 2A). This data allowed us to draw two conclusions, first, radiation-induced TGFβ signaling does protect irradiated cells from genomic instability and second, endogenous TGFβ plays a role in controlling/regulating centrosome status in S1 cells.

Task 2: If the frequency of genomically unstable colones is increased in any of the treated colonies compared to the sham-treatment group, then analyze the correlation between genomic instability and specific microenvironment markers.

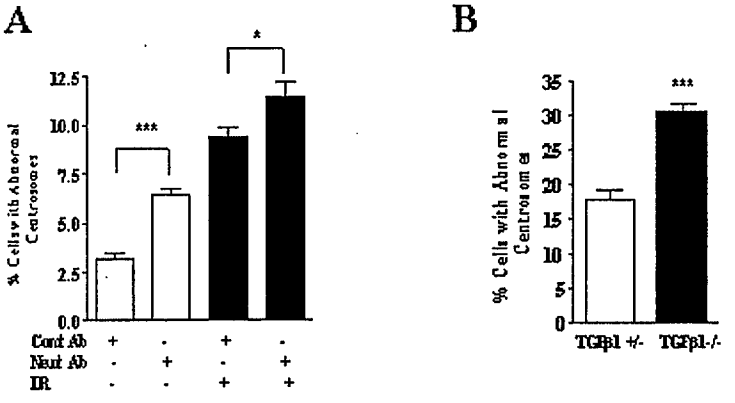

Figure 2. **(A)** HMECs were Sham (white) or 2Gy irradiated (black) shortly after plating and grown in the presence of TGF-β neutralizing Ab or control IgG. After 6 days centrosome status was assessed by γ-tubulin staining. **(B)** Centrosome status of TGFβ1+/- (white) and TGFβ1-/- (black) keratinocytes was evaluated by γ-tubulin staining in confluent monolayers.

We found that cell extracts of irradiated monolayer cultures have ~30% less E-cadherin than unirradiated cells by Western blot analysis. In order to investigate if irradiated cells produced a soluble factor which would result in reduced E-cadherin, we grew unirradiated cells in the presence of conditioned medium (CM) from irradiated cells. Unirradiated cells were grown in CM from unirradiated cells as a control. E-cadherin was reduced in unirradiated cells grown in CM from irradiated cells implying that a soluble factor was involved in the irradiated phenotype.

We know from our other studies and examples in the literature that TGF-β treatment can cause downregulation of E-cadherin in HMECs. In order to investigate the possible role of TGF-β in the downregulation of E-cadherin in the irradiated cells we grew the cells in the presence of TGF-β neutralizing antibodies. The neutralizing antibodies reversed the reduction of E-cadherin in the irradiated cells, suggesting that TGF-β is involved in the irradiated phenotype. With this data in

mind and the CM results described above we conducted an additional experiment in which we grew unirradiated cells in the presence of CM from irradiated cells +/- TGF-β neutralizing antibodies. The TGF-β neutralizing antibodies reversed the reduction in E-cadherin protein caused by the irradiated CM. These results suggest that TGF-β is involved in establishment of the irradiated phenotype.

Thus studies to test whether genomical instability correleated with loss of E-cadherin were made redundant by the findings noted above. That is, while we have found that exogenous and endogeneous TGFβ augments the loss of E-cadherin and promotes the expression of a mesenchymal phenotype in monolayer cultures, it's addition clearly suppressed centrosome aberrations. Thus genomic instability as measured by centrosomes is uncoupled from the mesenchymal phenotype that is driven by TGFβ exposure of irradiated HMEC. Recent publication by the Bissell lab have also shown that while genomic instability and EMT induced by MMP-2 can be functionally linked [Radisky, 2005], it is uncoupled when TGFβ drives such phenotypic changes (unpublished).

Interestingly, one might conclude that TGFβ's action as both a tumor suppressor and promoter is encapsulated in these experiments.

Aim 2

Task 1: To determine whether co-culture of radiation-chimeric populations consisting of differentially marked irradiated and unirradiated cells confer the irradiated phenotype on colonies arising from unirradiated cells.

To test this, we attempted to develop a protocol to differentially label HMEC with 'CellTracker' dyes (Molecular Probes, Eugene OR) which passively diffuse into live cells. Once inside, the non-fluorescent compounds are cleaved by intracellular enzymes to yield highly fluorescent dyes. After labeling, the external dyes are removed by changing the medium. Of the two types of CellTracker dyes, a thiol or amine reactive, only the thiol group was retained in this cell type. In addition, loading the mammary epithelial cell with dye was not completely non toxic. In cells already damaged by ionizing radiation, loading cells reduced viability. Therefore, in the experiments described below, only normal cells were labeled with thiol reactive CellTracker dyes.

The original experimental approach was to start by subculturing the cells. While the cells were in suspension, they were divided into two groups. The normal group was stained for 30 minutes with a CellTracker dye; the IR group was irradiated with 2 Gy of x-rays. The cells were then plated onto multi-chamber slides B one chamber normals only, a second chamber IR only, and two chambers with varying amount of both types. We then analyzed changes in E-cadherin levels between cells and changes in centrosome number. This experimental design turns out to have several disadvantages. One, by labeling cells with dye at the time of seeding, the cells go through many rounds of cell division causing the dye to be diluted fairly rapidly. This limits the duration of the experiment to only 3 or 4 days. Two, by mixing normal and irradiated cells together at time zero, one is observing both the recovery from being subcultured, the transition to forming colonies, and bystander interactions. Three, in mixed population experiments where the two groups are dispersed amongst each other, it is more difficult to resolve who is influencing whom. These problems can be remedied by change in experimental protocol.

In our new design, normal cells will be seeded at a low density and allowed to grow into small to medium colonies. The cells can then be labeled with CellTracker dye and the medium changed to clear the excess dye. Then irradiated cells will be plated at a moderate density in the same well and they will surround the normal colony. After, 4 days we will analyze changes in the normal

colonies as to e-cadherin levels and centrosome number. The controls will be to add unlabeled normal cells to the dye labeled normal colonies. This design should help retain the CellTracker dye since the normal labeled cells will proliferate less. In addition, the normal cells will be in colonies so perturbations due to recovery from subculturing should be minimal. The analysis will be easier since we can focus on changes within a colony of only normal cells.

Even a well thought out experimental approaches are still susceptible to the complexities of the cellular response. In this case, HMT3522 S1 HMEC form distinct colonies with highly polarized boundary cells. There was a high exclusion rate for a colony to allow a newcomer into the group. In addition, there is a long lag time between seeding the cells and rapid growth. The consequence was that the normal cells, seeded earlier, had a tremendous growth advantage over the irradiated cells. As a result, most of the colonies were pure normal cells. Nevertheless, there were colonies of mixed derivation but the influence irradiated cells (2 Gy) on their neighbors does not extrapolate easily from experiments using uniformly irradiated cultures.

As discussed above, E-cadherin staining and expression levels drop dramatically in uniformly irradiated cultures. In mixed cultures, the influence of the irradiated cell on the normal cell is not so clearly defined. In Fig. 3, is shown the situation of an HMEC island containing a minority of irradiated cells. In this case, the irradiated cells appear to express a "normal" level of E-cadherin. One hypothesis is that "bystander" effects can be both negative and positive. However, to better resolve the underlying interaction between normal and irradiated cells will require quantitative analysis of many mixed islands. This analysis requires the development of new software and is ongoing.

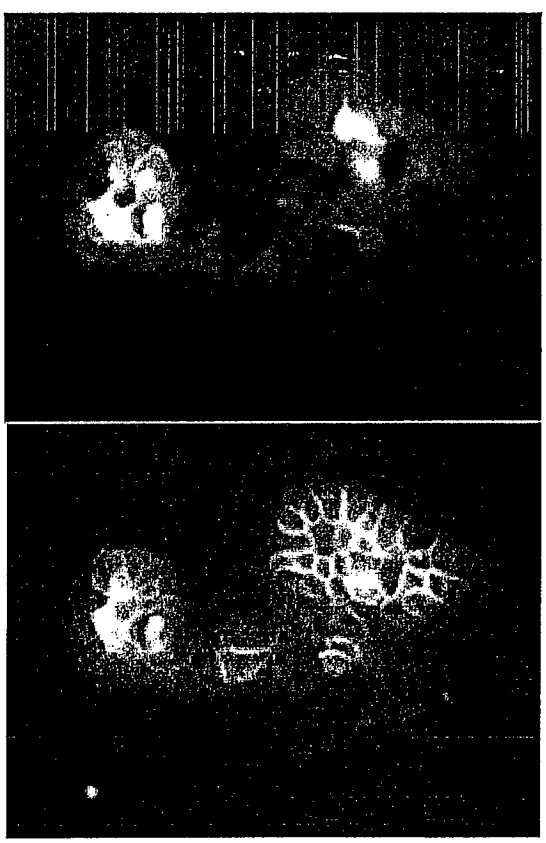

Figure 3 - The influence of irradiated cells on the expression levels of E-cadherin in normal HMEC. Normal cells were plated and grown for 2 days at which time they were labeled with CellTracker dye. On the third day cells irradiated with 2 Gy were added and the cultures were incubated for an additional 4 days. The top frame shows normal cells labeled with a fluorescent CellTracker dye (irradiated cells are unlabeled). In the bottom frame, cells are stained with a fluorescent antibody to E-cadherin.

Task 2: Determine whether radiation chimeric colonies convey the irradiated phenotype to progeny of unirradiated cells.

Notably, the conditioned media experiments outlined under Task 2 above, indicate that indeed a secreted factor produced by irradiated cells, namely TGFβ, can influence the expression of a critical cell-cell adhesion molecule, E-cadherin.

Aim 3

Task 1: To determine whether the loss of E-cadherin immunoreactivity and protein abundance is a function of hypermethylation of the E-cadherin gene in populations arising from sham, irradiated, TGF-β treated, or dual-treated cells.

Graff et al. (1997) has mapped the patterns of nine CpG island methylations for the E-cadherin gene

in normal and neoplastic cells. To establish conditions for methylation specific PCR (MSP) of the E-cadherin gene genomic DNA was isolated from MDA-MB-231 and MCF-7 cell lines. MDA-MB-231 cells do not express E-cadherin due to silencing of the E-cadherin gene by methylation whereas MCF-7 express E-cadherin. The genomic DNA was subjected to bisulfite modification and PCR amplication was carried out using primer pairs previously described for three separate CpG islands in the E-cadherin gene (Graff et al. 1997). One of the primer pairs (A2) amplifies a region in both cell lines that is methylated while the other two primer pairs (IS2 and IS4) amplify regions which are methylated only in MDA-MB-231 cells. As expected we found that the A2 region was methylated in both cell lines and when MSP was carried out using primers to the IS2 and IS4 regions only the MDA-MB-231 DNA was methylated. We have successfully established this technique in our lab and have both positive and negative controls available when evaluated our sample genomic DNA.

Silencing of the E-cadherin gene would result in reduction of the E-cadherin mRNA. Before carrying out MSP we carried out quantitative RT-PCR using a Lightcycler real-time PCR machine (Roche) for the E-cadherin message on RNA isolated from monolayer cultures treated with +/-2Gy and/or +/- TGF-β. Cells treated with TGF-β or TGF-β and 2Gy irradiation showed a marked reduction in E-cadherin message, compared to untreated cells, which correlated with the decreased E-cadherin protein found in these cells. While RNA from 2Gy irradiated cells showed only a slight decrease in E-cadherin mRNA levels compared to untreated cells. Since E-cadherin mRNA levels were altered we followed through with MSP for the E-cadherin gene.

Genomic DNA was recovered from cells grown in monolayers or embedded in rBM which had been treated with +/-2Gy -irradiation and/or +/-TGF-β. MSP was carried out using the primers and controls described above. Treatments did not change the methylation patterns for the sites we examined suggesting that methylation of these three sites in E-cadherin promoter is not the mechanism by which E-cadherin protein abundance is reduced in the irradiated phenotype.

We knew from the MSP experiments that the E-cadherin promoter was not differentially methylated between treatment groups. Instead of approaching this experiment with the idea of demethylating the E-cadherin gene and inducing its expression we came from a different point of view and asked if methylation played a role in the irradiated phenotype. MDA-MB-231 cells were used as a control for determining toxicity and efficiency of the 5azadC treatment. It was determined that 2.5 M 5azadC was not toxic to the cells and was sufficient to induce E-cadherin expression.

We carried out preliminary experiments in which TGF-β, irradiated, or dual treated cells were grown in the presence or absence of 2.5 M 5azadC. E-cadherin protein abundance was monitored via Western blot analysis. In the absence of 5azadC the cells displayed the same pattern of E-cadherin abundance that was described previously. There is a reduction in E-cadherin protein after 2Gy irradiation and E-cadherin abundance is decreased further when cells are grown in the presence of TGF-β +/- 2Gy. When 5azadC was present under the same conditions there was a slight reversal or increase in 2Gy and dual treated cells, but TGF-β cells displayed an additional reduction in E-cadherin abundance.

Task 2: To determine whether the E-cadherin gene expression in irradiated cells is affected by changes in histone acetylation.

These experiments were proposed as an alternative to methylation but were not executed since the above data were promising and due to timing and personnel constraints.

Reportable Outcomes

Presentations by PI that Referred to Data Generated by this Grant

"Persistent Epithelial Phenotypic Changes Induced by Radiation: Evidence of Epithelial-Mesenchymal Transition that may Promote Neoplastic Progression" 16th Annual NASA Space Radiation Investigators' Workshop, May 15-18, 2005 in Port Jefferson, New York

"Modulation of Mammary Stromal-Epithelial Interactions During Radiation Carcinogenesis" Keystone Symposia on The Role of Microenvironment in Tumor Induction and Progression, Banff, Canada, February 8, 2005.

"Mechanisms and Consequences of TGFß Activation in Response to Ionizing Radiation" Department of Radiation Oncology, University of Michigan, Ann Arbor, January 20, 2005.

"Extracellular Controls of Radiation Response" DARPA Workshop on Radiation Protection, Arlington, VA, December 14, 2004.

"Unlikely Partners: p53 and TGF-β1", The International p53 Workshop, Dunedin, New Zealand, November 7, 2004.

"TGFβ Regulation of Estrogen Receptor α Positive Cells in Mammary Gland Development", Westmead Institute for Cancer Research, University of Sydney at the Westmead Millennium Institute, Department of Translational Oncology, Westmead, Australia, November 11, 2004.

"TGF-β Regulation of Hormone Response in the Mammary Gland" The Endocrine Society, New Orleans, LA, June 19, 2004.

"Interaction of Cell and Tissue Stress Responses" 3rd International Workshop on Space Radiation Research, Port Jefferson, NY, May 16-20, 2004.

"Non-Mutagenic Actions of Radiation in Mouse and Human Mammary Carcinogenesis", Keystone Symposia on Mouse Models of Cancer, February, 21, 2004.

"Regulation of Cell Fate Decision by TGF-β Following DNA Damage", Graduate Program in Cell and Molecular Biology, Colorado State University, Ft. Collins, CO, February 27, 2004.

"Basic Studies of Solid Tumor Cancer Risk Assessment" Workshop on Solid Cancer Risks from Space Radiation, Houston, TX, January 26, 2004.

"Non-Mutagenic Actions of Radiation in Mouse and Human Mammary Carcinogenesis", Keystone Symposia on Mouse Models of Cancer, February, 21, 2004.

"TGF-β's Role in Mammary Gland Development and Carcinogenesis", 24th Congress of the International Association for Breast Cancer Research, Sacramento, CA, November 4, 2003.

"TGF-β Regulation of Cell Fate Decisions Following DNA Damage", Ludwig Institute for Cancer Research Uppsala, Sweden, September 26, 2003.

"The Role of TGF-β in Radiation Response", Federation of European Cancer Societies, Copenhagen, Denmark, September 23, 2003.

"Extracellular Signaling in Radiation Responses", International Congress of Radiation Research, Brisbane, Australia, August 19, 2003.

"Of Mice and Women: Modeling Radiation Carcinogenesis", Life Sciences Division, Oak Ridge National Laboratory, August 4, 2003.

"Mechanisms and Consequences of TGF-β1 Activation in Mammary Gland Development and Carcinogenesis", University of Puerto Rico Cancer Center and Departments of Biochemistry and Physiology, June 9, 2003.

"Interaction Between Tissue and Cellular Stress Responses: TGF-β1 is a Key Mediator of the DNA Damage Response", 14th Space Radiation Health Investigators Workshop, Houston, TX,

April 28, 2003.

"Mechanisms and Consequences of TGF-β1 Activation in the Mammary Gland: Response to Hormones and DNA Damage", UC Davis Cancer Center, March 6, 2003.

"TGF-β's Role in DNA Damage Responses", Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, National Institutes of Health, Bethesda, MD, February 26, 2003.

"The Role of TGF-β in DNA Damage Response", AACR Special Conference: *The TGF-β Superfamily: Roles in the Pathogenesis of Cancer and Other Diseases*, La Jolla, CA, January 15-19, 2003.

Poster and Meeting Participation

Erickson, A.C., Gupta, R., Parvin, B. and Barcellos-Hoff, M.H. TGFβ1 Protects Human Mammary Epithelial Cells from Radiation-Induced Centrosome Amplification. DOE Low Dose Radiation Workshop, October 27-29, 2003.

Gupta, R., Erickson, A.C., Parvin, B. and Barcellos-Hoff, M.H. TGFβ1 Protects Human Mammary Epithelial Cells from Radiation-Induced Centrosome Amplification. American Society for Cell Biology, San Francisco, December 9-12, 2003.

Erickson, A.C., Chou, W.S., Henshall-Powell, R.L., Bissell, M.J. , Barcellos-Hoff, M.H. Radiation alters cytoskeletal association of E-cadherin in TGF-β treated human mammary epithelial cells. American Society for Cell Biology, San Francicso, CA, December 14-18, 2002.

Erickson, A.C., Chou, W.S., Henshall-Powell, R.L., Bissell, M.J. , Barcellos-Hoff, M.H. The progeny of irradiated human mammary epithelial cells exhibit a distinct phenotype in response to transforming growth factor-β1. ERA of HOPE, Orlando, FL, September 25-28, 2002.

Personnel Receiving Support

Mary Helen Barcellos-Hoff, Ph.D.: Principle Investigator

Rick Schwarz, Ph.D.: Collaborator

A. Erickson, Ph.D.: Worked on this project until 9/04 but supported by DOD BCRP Training grant to M.J. Bissell

J. Kirshner, Ph.D.: Postdoctoral fellow

R. Gupta: Undergraduate research assistant

W. Chou: Research technician

Conclusions

Our current studies regarding radiation-induced phenotype and genomic instability support the widly acknowledged dual role of TGFβ in cancer [14]. In mouse models overexpression of active TGFβ suppresses tumorigenesis [15], but converts to a tumor promoting role in late carcinogenesis in skin, mammary gland and colon [16-18]. In response to radiation TGFβ likewise appears to protect against genomic instability generated by centrosome amplification, while promoting phenotypic neoplastic progression. Our future studies will seek to define the molecular mechanism by which radiation-induces centrosome amplification and that by which TGFβ regulates it.

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